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SNP risk profiling in childhood acute lymphoblastic leukemia

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Leukemia - advance online publication, 18 March 2011

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Background

- cure rate for childhood acute lymphoblastic leukemia (ALL) approaches 80-85%
- need for personalized therapy
- SNPs - key determiners of inter-individual differences in treatment efficacy and toxicity
- ALL is a model disease for exploring the impact of genetic variation due to well-characterized cytogenetics, drug response pathways, and precise monitoring of minimal residual disease

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Background

- investigate drug metabolism
- determine drug responders versus non-responders
- optimize drug dosing

⇒ genotype patients for known pharmacogenetic markers

- 50 VIP SNPs – literature curated

Davidson ML, Dalhoff K, Schmiegelow K.
 Pharmacogenetics influence treatment efficacy in childhood acute lymphoblastic leukemia. *J PediatrHematolOncol*2008; 30: 831-849.

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SNP selection

Literature curation
& core gene list

→

*First-order protein-protein interaction partners, and physical-interaction derived disease modules

*Pathways (Reactome, KEGG and PharmKB pathways)
*miRNA target

→

Filter SNPs
&
Bait design

→

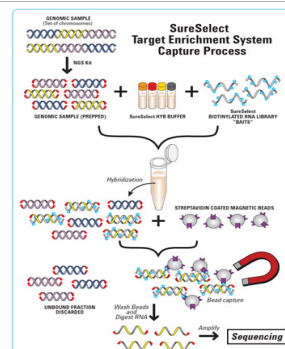
List	Number of SNPs
SNP list	864
Core gene list	7,190
Extended gene list	14,714
Pathway	4,422
Drug list	5,464
miRNA	3,672
Total SNPs	25,602

Tiling baits	
Genomic	CDKN2A, GSTM1, GSTT1
Exon	ETV6, MITF, OPRM1

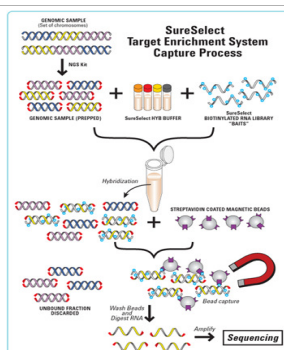
Choice of method

- 25,000 clinically relevant SNPs
 - commercially available SNP chips are designed to explore non functional variations across the genome
 - costs of custom-made approaches are too high for implementation in clinical settings
 - or minimum order quantity too high
- => multiplexed targeted sequencing

SureSelect Target Enrichment



SureSelect Target Enrichment



Expensive

Expensive

Multiplexing reduces cost


- 4 nt long barcodes (red) can be used to label different samples when ligating sequencing adaptors (blue)



- by multiplexing several samples together cost can be reduced
- 1 SureSelect Target Enrichment kit & 1 sequencing lane per multiplex

Bait design – childhood ALL samples

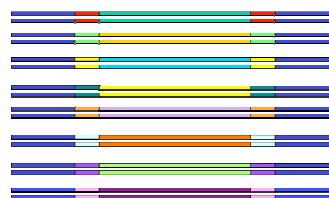


- each SNP targeted by 2 baits with 50% overlap
- 
- physical and cross-hybridization properties explored with OligoWiz, BLAST and SeqMap
 - cross-hybridization
 - self-folding
 - extreme GC content
 - baits targeting highly variable regions
 - additional baits tiling exons of ETV6, MTAP and OPRM1 and entire genomic regions of CDKN2A, GSTM1 and GSTT1

Childhood ALL samples



- multiplexing 8 samples together, each with an individual barcode



- 6 lanes on Illumina GAIIx => 48 samples
- custom designed baits

Childhood ALL samples - results



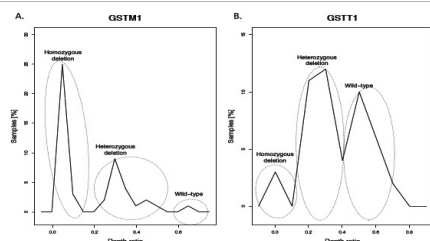
- on average 94% of the targeted SNPs covered by $\geq 1x$
- 73% of those achieve at least 10x depth
- mean depth for the covered variations was 23x across all the samples
- mean depth for exons and genomic regions – 32x

Genotype validation



- patients were previously genotyped for 7 of the tested SNPs by allelic discrimination and by multiplexing PCR
- > the concordance for those SNPs was between 91-100 %
- two common gene deletions have been tested – GSTT1 and GSTM1
- > concordance 100%

Genotype validation – gene deletions



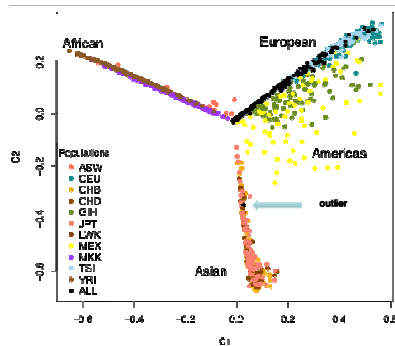
Depth ratio was calculated from the number of reads in the targeted genomic region normalized by size of the region and total number of reads for the sample. Results were validated for 42 of the 48 samples using multiplexing PCR and showed 100% concordance.

Childhood ALL project



- 233 samples from childhood ALL patients (collected after first remission)
- information about age, WBC (white blood count), risk group classification, MRD day 29 (minimum residual disease), EFS (event free survival), type of event
- Methotrexate (MTX) courses
- Other...

Patient ethnicity



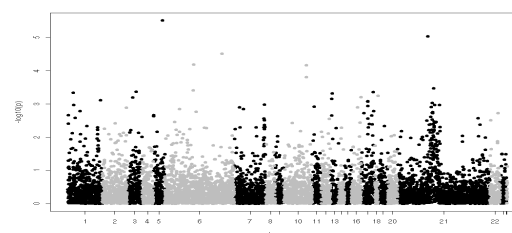
- Based on ~ 4,200 SNPs overlapping with HapMap dataset

First results



Cases: relapse Controls: complete remission

After QC: 20 cases vs 165 controls
7,363 SNPs

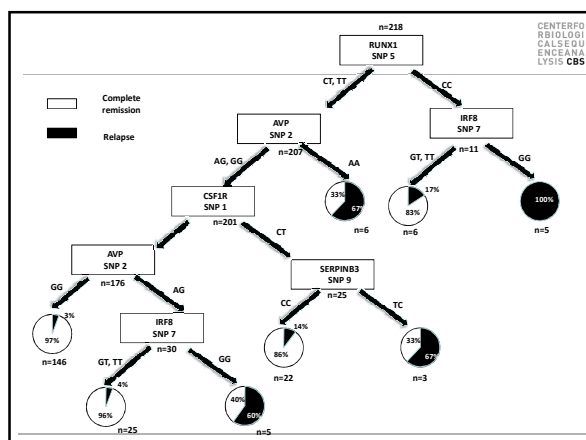
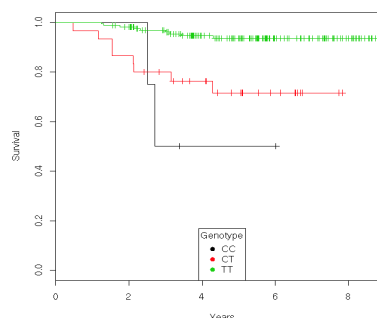


Relapse vs Complete remission



SNP	Chromosome	P-val	Gene	Consequence	List	Comments
SNP 1	Chr 5	3.068e-06	CSF1R	Splice site miRNA target site - DC	First druglist	Cytarabine, Etoposide, Dexamethasone
SNP 2	Chr 21	9.236e-06	RUNX1	Within non-coding gene	PharmGKB genes	Clinical outcome
SNP 3	Chr 6	3.095e-05	WDR46 / PFDN8	Non-synonymous coding	PharmGKB genes	Pharmacodynamics
SNP 4	Chr 6	6.576e-05	HLA-DRB1	Within non-coding gene	Core, Extended	Pharmacogenetics
SNP 5	Chr 10	6.893e-05	ABCC2	Non-synonymous coding	Core, Extended, Pathway	Transport VCR, DOX, DNR, MTX, EPI Path: ABC transporters, Doxorubicin (Cancer PD), Methotrexate Pathway
SNP 6	Chr 10	0.0001574	ABCC2	Non-synonymous coding	Core, Extended, Pathway	Transport VCR, DOX, DNR, MTX, EPI Path: ABC transporters, Doxorubicin (Cancer PD), Methotrexate Pathway
SNP 7	Chr 21	0.0003362	RUNX1	Within non-coding gene	PharmGKB genes	Clinical outcome
SNP 8	Chr 6	0.0003925	HLA-DRB1	Within non-coding gene	Core, Extended	Pharmacogenetics
SNP 9	Chr 3	0.0004295	TOPBP1	Non-synonymous coding	Core, Extended	Drug_target

Top SNP – survival curve



Future work



- SNPs in the same pathways, protein complexes etc.
- SNP interactions
- Neural network classification
- Validation in another cohort



For more details on the method see:

Wesolowska A. et al. Cost-effective multiplexing before capture allows screening of 25,000 clinically relevant SNPs in childhood ALL. *Leukemia, in press.*
<http://tinyurl.com/5rWSCME>